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PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53 (c)

Docket Number	P-16438	Type a plus sign (+) inside this box -->	+

INVENTOR(s)/APPLICANT(s)

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60/557347
032904

TITLE OF THE INVENTION (280 characters max)

USES OF MELANOCORTIN-4 RECEPTOR (MC4R) AGONIST PEPTIDES ADMINISTERED BY CONTINUOUS INFUSION

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ENCLOSED APPLICATION PARTS *(check all that apply)*

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	Drawing(s)	Number of Sheets			Other (Specify)	

METHOD OF PAYMENT *(check one)*

			PROVISIONAL FILING FEE AMOUNT ($)
	A check or money order is enclosed to cover the Provisional filing fees		
X	The Commissioner is hereby authorized to charge filing fees and credit Deposit Account Number:	05-0840	$160.00

The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government.

[X] No.

[ ] Yes, the name of the U.S. Government agency and the Government contract number are:

Respectfully submitted,
SIGNATURE Paula K. Davis

Date 03/29/04

REGISTRATION NO. *(if appropriate)* 47,517

TYPED or PRINTED NAME PAULA K. DAVIS

[ ] Additional inventors are being named on separately numbered sheets attached hereto

PROVISIONAL APPLICATION FOR PATENT FILING ONLY

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Queen Thomas
Printed Name

Queen Thomas
Signature

USES OF MELANOCORTIN-4 RECEPTOR (MC4R) AGONIST PEPTIDES ADMINISTERED BY CONTINUOUS INFUSION

BACKGROUND OF THE INVENTION

The melanocortin-4 receptor (MC4R) is a G-protein coupled receptor (GPCR). MC4R mediates a signal that it receives from the endogenous melanocortin stimulating hormones (MSH) and the agouti related protein peptide (AGRP) in the hypothalamus. The former peptides are processed from a proopiomelanocortin (POMC) precursor protein produced by the neurons in the arcuate nucleus of the hypothalamus. Those peptides are competitive full agonists for the MC4 receptor. Conversely, AGRP is reported to be either a competitive antagonist or an inverse agonist at the same receptor. This endogenous messenger is also produced and released by neurons in the hypothalamus but distinct from those synthesizing POMC. Together, the melanocortin system is part of the neuronal hypothalamic network regulating energy balance.

It has been proposed that during physiological states characterized by a negative energy balance, AGRP signaling is enhanced and POMC signaling is reduced. Further, those responses are thought to participate in correcting the negative energy balance. Specifically, AGRP signaling would dominate over MSH signaling, resulting in enhanced appetite and decreased energy expenditure via decreased activity of the sympathetic nervous system.

Etiology and pathophysiology of obesity remains a subject of intense study. There are rare examples of obese individuals and obese rodents with mutations of MC4R or POMC genes. Over-expression of an AGRP transgene will also present an obese mouse. There are no examples of over-expression of POMC producing a lean phenotype.

This raises the possibility that MC4R may be desensitized during continuous exposure to its agonists. Indeed, there are many examples of GPCRs that are down regulated by chronic exposure to their agonists.

Daily peripheral administration of the MSH agonist melanotan II (MT-II) for at least one week decreases weight gain in rodents, indicating that a peripheral injection of the peptide will trigger the MC4 receptor in the hypothalamus and that a lean phenotype can be realized. Further, such studies suggest no desensitization after intermittent administration. Because those peptides have a short half-life and were only administered intermittently, it follows that the receptor was also only infrequently occupied and that may have prevented any down regulation or desensitization.

A need exists to find an agonist capable of triggering the MC4 receptor, capable of being administered such that the receptor remains occupied, but without down regulation or desensitization of the receptor. Meeting this need will provide a means to induce weight loss and overcome obesity, a disease that has major debilitating effects on the body.

SUMMARY OF THE INVENTION

The present invention provides a method of inducing weight loss in a patient, comprising continuous infusion of an effective amount of an MC4R agonist peptide into the patient. Additionally, the present invention provides a method of treating obesity in a patient, comprising continuous infusion of an effective amount of an MC4R agonist peptide into the patient.

The instant invention demonstrates that when the same mass of an MC4R agonist peptide is delivered to patients using two different methods: (1) a single daily bolus subcutaneous administration, or (2) by continuous subcutaneous infusion, the peptide is much more effective when administered continuously than intermittently. Those data suggest that the MC4 receptor can be continuously occupied with an agonist without down regulation or desensitization.

Moreover, a low rate of infusion, for example approximately 2 μg/hr of Ac-D-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2 infused into the subcutaneous environment, is sufficient to overcome metabolism and dilution of the peptides to

successfully bind the hypothalamic receptor in quantities that would overcome competition by AGRP.

Furthermore, delivery of the peptide via continuous infusion allows the MC4 receptor to remain continuously occupied. Importantly, this overcomes problems associated with bolus injections. For instance, due to short half-life of the MC4R agonist peptide, shortly after a bolus injection is made, the peptide degrades, leaving the receptor open for antagonists or inverse agonists to occupy. Occupation by an antagonist or inverse agonist may not induce weight loss; conversely, it may induce weight gain. Yet, with continuous infusion of the MC4R agonist peptide, the receptor remains occupied with the agonist. Additionally, potential side effects caused by bolus injections, such as penile erection, may be avoided.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of the present invention, as disclosed and claimed herein, the following terms are as defined below.

"Continuous infusion" of an MC4R agonist peptide refers to controlled parenteral delivery of the peptide to a patient for an extended period of time. Administration of the peptide may be accomplished by, but is not limited to, delivery via pump, depot, suppository, pessary, transdermal patch or other topical administration (such as buccal, sublingual, spray, ointment, creme, or gel) using, for example, subcutaneous, intramuscular, intraperitoneal, intravenous, intracerebral, or intraarterial administration.

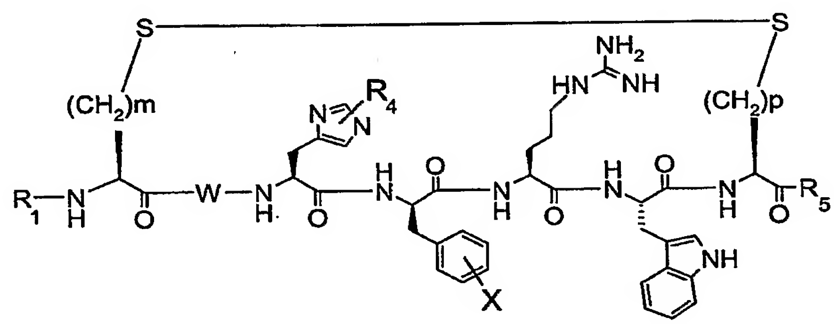
A pump delivering the MC4R agonist peptide into the body may be implanted in the patient's body. Alternatively, the patient may wear a pump externally, being attached to the patient's body via catheter, needle, or some other connective means. Any pump that is suitable for the delivery of pharmaceuticals to a patient may be used. Examples include pumps such as those disclosed in US Pat. No. 6,659,982.

A depot is a biocompatible polymer system containing the MC4R agonist peptide and delivering the peptide over time. Examples include microspheres, microcapsules, nanoparticles, liposomes, a hydrogel, or other polymeric implants. Preferred periods for delivery of agonist by depot include one week, two weeks, and one month periods. If needed, another depot will be delivered to the patient for continued delivery of peptide.

Engineering the MC4R agonist peptide to have a prolonged half-life will also result in continuous delivery of the MC4 receptor agonist to the receptor. Such modifications include conjugations with larger proteins such as albumin, antibody and antigen or chemical modifications that may increase half-life by linking fatty acids, polyethelene glycol (PEG) polymers, and other agents.

An "MC4R agonist peptide" utilized in the instant invention includes any agonist peptide which has affinity for the MC4 receptor. Examples include, but are not limited to, MC4R agonists disclosed in the following art: US Pat. No. 5,674,839; WO 01/52880; WO 03/006604; WO 00/36136; WO 01/00224; WO 01/13112; WO 00/58361; US Pat. No. 6,613,874; WO 02/26774; WO 99/54358; WO 01/74844; WO 02/18437; WO 98/27113; WO 01/05401; US Pat. No. 5,731,408; and WO 01/85930.

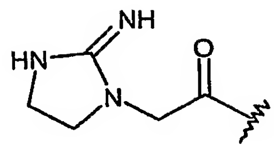
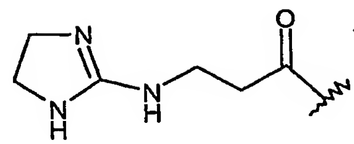
In a preferred embodiment, the MC4R agonist peptide is represented by the following Structural Formula I:

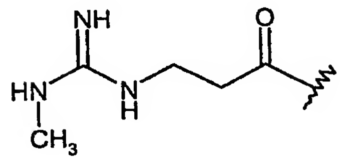
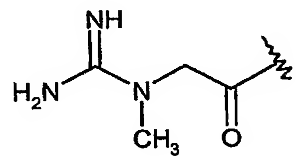


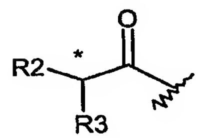
and pharmaceutically acceptable salts thereof, wherein

W is a single bond, Glu, Gln, Asp, Asn, Ala, Gly, Thr, Ser, Pro, Met, Ile, Val, Arg, His, Tyr, Trp, or Phe;

R_1 is -H, $-C(O)CH_3$, $-C(O)(CH_2)_{1-4}CH_3$, $-NH-C(NH)NH_2$, Tyr-βArg, gluconoyl-Tyr-Arg, Ac-Dab, Ac-Dap, N-succinyl-Tyr-Arg, N-propionyl, N-valeryl, N-glutaryl-Tyr-Arg, N-butyryl,

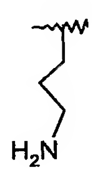
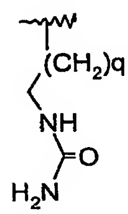
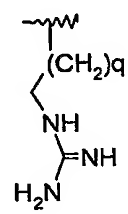
, ,

, , or

, wherein

R_2 is -H, $-NH_2$, $-NHC(O)CH_3$, $-NHC(O)(CH_2)_{1-4}CH_3$, Tyr, or -NH-Tyr-$C(O)CH_3$;

R_3 is C_1-C_4 straight or branched alkyl, Ser, Ile, Arg,

  or ;

q is 0, 1, 2, or 3;

m is 1 or 2;

p is 1 or 2;

R_4 is -H, $-CH_3$, or $-(CH_2)_{1-3}CH_3$;

X is -H, -Cl, -F, -Br, methyl, or methoxy; and

R_5 is $-NH_2$, -OH, glycinol, -Ser-Pro-NH_2, -Lys-Pro-NH_2, -Ser-OH, -Ser-Pro-OH, -Lys-Pro-OH, -Arg-Phe-NH_2, -Glu-NH_2, -NHR, or -OR, where R is $-CH_3$ or $-(CH_2)_{1-3}CH_3$.

In another preferred embodiment, the MC4R agonist peptide is represented by compounds of the Structural Formula I wherein W is Glu or a single bond; R_4 is -H or $-CH_3$; X is -H, -Cl, -F, or -Br; and R_5 is $-NH_2$ or -OH.

In another preferred embodiment, the MC4R agonist peptide is represented by the compound of the Structural Formula I wherein m is 1; p is 1; W is Glu; R_1 is Ac-D-Arg; R_4 is -H; X is -H; and R_5 is $-NH_2$.

Furthermore, MC4R agonist peptides of the instant invention include those compounds having the following structures:

1: cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2

2: Ac-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2

3: Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2

4: Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-OH

5: Ac-Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2

6: Ac-Arg-cyclo[Cys-Glu-His-(4Cl-D-Phe)-Arg-Trp-Cys]-NH_2

7: Ac-Tyr-Arg-cyclo[Cys-Glu-His-(4Cl-D-Phe)-Arg-Trp-Cys]-NH_2

8: Ac-Tyr-Arg-cyclo[Cys-Glu-His-(4F-D-Phe)-Arg-Trp-Cys]-NH_2

9: Ac-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

10: Ac-D-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

11: Ac-Orn-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

12: $(H_2N)C(HN)HNCH_2CH_2CH_2C(O)$-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2.

13: Tyr-Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2

14: Ac-Tyr-Arg-cyclo[Cys-Glu-His-(4Br-D-Phe)-Arg-Trp-Cys]-NH_2

15: Ac-cyclo[Cys-Glu-His-(4Br-D-Phe)-Arg-Trp-Cys]-NH_2

16: $(H_2N)C(HN)HNCH_2CH_2CH_2CH_2C(O)$-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

17: Ac-hArg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

18: Ac-Tyr-hArg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

19: Ac-Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2

20: Ac-cyclo[Cys-Glu-His-(4F-D-Phe)-Arg-Trp-Cys]-NH_2

21: Ac-Lys(ipr)-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

22: Ac-Tyr-Lys-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

23: Ac-Tyr-Arg-cyclo[Cys-Glu-(1Me-His)-(4Br-D-Phe)-Arg-Trp-Cys]-NH_2

24: Ac-Tyr-Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-OH

25: Ac-Tyr-D-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

26: Ac-Lys-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

27: $(H_2N)C(HN)HNCH_2CH_2C(O)$-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

28: Ac-Cit-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

29: Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

30: Tyr-Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-OH

31: Ac-Tyr-Cit-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

32: Arg-cyclo[hCys-His-D-Phe-Arg-Trp-hCys]-NH_2

33: Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

34: cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-OH

35: Ac-cyclo[Cys-Lys-His-D-Phe-Arg-Trp-Cys]-NH_2

36: Ac-cyclo[Cys-Leu-His-D-Phe-Arg-Trp-Cys]-NH_2

37: Ac-cyclo[Cys-Gln-His-D-Phe-Arg-Trp-Cys]-NH_2

38: Ac-cyclo[Cys-Asp-His-D-Phe-Arg-Trp-Cys]-NH_2

39: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Ser-Pro-NH_2

40: Ac-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Ser-Pro-NH_2

41: Ac-nLeu-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Ser-Pro-NH_2

42: 3-(4-hydroxyphenylpropionyl)-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

43: Ac-Tyr-Arg-cyclo[hCys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

44: Ac-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Lys-Pro-NH_2

45: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Lys-Pro-NH_2

46: 3-(4-methylbenzoyl)propionyl-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

47: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Arg-Phe-NH_2

48: Ac-Tyr-Arg-cyclo[Cys-Gly-His-D-Phe-Arg-Trp-Cys]-NH_2

49: Ac-Tyr-Val-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

50: Ac-Tyr-Arg-cyclo[Cys-His-His-D-Phe-Arg-Trp-Cys]-NH_2

51: Ac-Tyr-Arg-cyclo[Cys-Ile-His-D-Phe-Arg-Trp-Cys]-NH_2

52: Ac-Tyr-Arg-cyclo[Cys-Met-His-D-Phe-Arg-Trp-Cys]-NH_2

53: Ac-Tyr-Arg-cyclo[Cys-Phe-His-D-Phe-Arg-Trp-Cys]-NH_2

54: Ac-Tyr-Arg-cyclo[Cys-Pro-His-D-Phe-Arg-Trp-Cys]-NH_2

55: Ac-Tyr-Ser-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

56: Ac-Tyr-Arg-cyclo[Cys-Ser-His-D-Phe-Arg-Trp-Cys]-NH_2

57: Ac-Tyr-Arg-cyclo[Cys-Thr-His-D-Phe-Arg-Trp-Cys]-NH_2

58: Ac-Tyr-Arg-cyclo[Cys-Trp-His-D-Phe-Arg-Trp-Cys]-NH_2

59: Ac-Tyr-Arg-cyclo[Cys-Val-His-D-Phe-Arg-Trp-Cys]-NH_2

60: Ac-Tyr-Arg-cyclo[Cys-Glu-His-(4Me-D-Phe)-Arg-Trp-Cys]-NH_2

61: Ac-Tyr-Arg-cyclo[Cys-Glu-His-(4OMe-D-Phe)-Arg-Trp-Cys]-NH_2

62: Ac-Tyr-Arg-cyclo[Cys-Glu-(1Me-His)-D-Phe-Arg-Trp-Cys]-NH_2

63: Ac-Tyr-Arg-cyclo[Cys-Glu-(3Me-His)-D-Phe-Arg-Trp-Cys]-NH_2

64: N-methyl-Tyr-Arg-cyclo[Cys-Met-His-D-Phe-Arg-Trp-Cys]-NH_2

65: Ac-Tyr-Arg-cyclo[hCys-His-D-Phe-Arg-Trp-hCys]-NH_2

66: Ac-Tyr-Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2

67: Ac-Tyr-Arg-cyclo[Cys-Gln-His-D-Phe-Arg-Trp-Cys]-OMe

68: Ac-cyclo[hCys-His-D-Phe-Arg-Trp-hCys]-NH_2

69: Ac-Arg-cyclo[Cys-Glu-(1Me-His)-D-Phe-Arg-Trp-Cys]-NH_2

70: Ac-Arg-cyclo[Cys-Glu-(1Me-His)-(4Cl-D-Phe)-Arg-Trp-Cys]-NH_2

71: Ac-Tyr-Arg-cyclo[Cys-Arg-His-D-Phe-Arg-Trp-Cys]-NH_2

72: Ac-Tyr-(L-β-hArg)-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

73: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Lys-Pro-OH

74: Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH-$(CH_2)_6$-NH_2

75: Ac-cyclo[Cys-Glu-His-(4Cl-D-Phe)-Arg-Trp-Cys]-NH_2

76: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Ser-Pro-OH

77: Ac-Tyr-Arg-cyclo[Cys-Ala-His-D-Phe-Arg-Trp-Cys]-NH_2

78: gluconoyl-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

79: Ac-Tyr-Arg-cyclo[Cys-Asn-His-D-Phe-Arg-Trp-Cys]-NH_2

80: Ac-Tyr-Arg-cyclo[Cys-Tyr-His-D-Phe-Arg-Trp-Cys]-NH_2

81: Ac-Tyr-Arg-cyclo[Cys-Glu-(5Me-His)-D-Phe-Arg-Trp-Cys]-NH_2

82: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH-$(CH_2)_6$-NH_2

83: Ac-Tyr-Arg-cyclo[Cys-Asp-His-D-Phe-Arg-Trp-Cys]-NH_2

84: Ac-Dab-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

85: Ac-Dap-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

86: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-2-(2-aminoethoxy) ethanol

87: N-succinyl-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

88: Tyr-Arg-cyclo[Cys-Gly-His-D-Phe-Arg-Trp-Cys]-NH_2

89: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-glycinol

90: Ac-nLeu-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

91: N-propionyl-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

92: Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

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98: N-glutaryl-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

99: Ac-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

100: Ac-Tyr-Arg-cyclo[Cys-Gln-His-D-Phe-Arg-Trp-Cys]-OH

101: N-butyryl-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

102: Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

103: Ac-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-OH

104: Ac-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

105: cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

106: Ac-Tyr-Arg-cyclo[Cys-Glu-(1Me-His)-(4F-D-Phe)-Arg-Trp-Cys]-NH_2

107: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-Glu-NH_2

108: Ac-Tyr-Arg-cyclo[Cys-Glu-(1Me-His)-(4OMe-D-Phe)-Arg-Trp-Cys]-NH_2

109: Ac-Tyr-Arg-cyclo[dCys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2

110: Ac-Tyr-Arg-cyclo[Cys-Glu-(1-Bzl-His)-D-Phe-Arg-Trp-Cys]-NH_2

111: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

112: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-alcohol

113: N-succinyl-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

114: Ac-Tyr-Arg-cyclo[Cys-Glu-(1Bom-His)-D-Phe-Arg-Trp-Cys]-NH_2

115: Ac-Tyr (Cit)-cyclo[Cys-Glu-(1Me-His)-D-Phe-Arg-Trp-Cys]-NH_2

116: Ac-Tyr-Arg-cyclo[hCys-Glu-His-D-Phe-Arg-Trp-hCys]-NH_2

117: Ac-(Cit)-cyclo[Cys-Glu-(1Me-His)-D-Phe-Arg-Trp-Cys]-NH_2

118: N-glutaryl-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-OH

119: Ac-Tyr-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-hCys]-NH_2

120: Ac-cyclo[Cys-Glu-(1Me-His)-D-Phe-Arg-Trp-Cys]-NH_2

As used herein, "alkyl" means straight chained or branched hydrocarbons, which are completely saturated.

The letter "D" preceding the above-mentioned 3-letter abbreviations, e.g., "D-Phe," means the D-form of the amino acid. When the single letter abbreviation is used for an amino acid, a "d" will precede the letter to designate the D-form of the amino acid (e.g., dF = D-Phe).

"*" means that both the D- and L- isomers are possible.

"Orn" refers to ornithine.

"hCys" refers to homocysteine.

"hArg" refers to homoarginine.

"Lys(ipr)" refers to Lysine(N-isopropyl).

"Cit" refers to citruline.

"nLeu" refers to norleucine.

"Dap" refers to diaminopropionyl.

"Dab" refers to diaminobutyryl.

Modified amino acids are indicated with parentheses around the amino acid and the modification thereto. For example, (4Cl-D-Phe) is a 4-chloro modification on the D-isomer of phenylalanine. With respect to moieties depicted in Structural Formula I, the single letter designations are as defined and do not refer to single letter amino acids corresponding to those letters.

The term "pharmaceutical" when used herein as an adjective means substantially non-deleterious.

"Pharmaceutically-acceptable salt" refers to salts of the compounds of the Structural Formula I that are substantially non-toxic to mammals. Typical pharmaceutically acceptable salts include those salts prepared by reaction of the compounds of the present invention with a mineral or organic acid or an organic or inorganic base. Such salts are known as acid addition and base addition salts, respectively. It should be recognized that the particular counterion forming a part of any salt of this invention is not of a critical nature, so long as the salt as a whole is pharmaceutically acceptable and as long as the counterion does not contribute undesired qualities to the salt as a whole.

A pharmaceutical "acid addition salt" is a salt formed by reaction of the free base form of a compound of formula I with a pharmaceutical acid, such as described in the Encyclopedia of Pharmaceutical Technology, editors James Swarbrick and James C. Boylan, Vol 13 (1996), "Preservation of Pharmaceutical Products to Salt Forms of Drugs and Absorption". Specific salt forms include, but are not limited to the: acetate, benzoate, benzenesulfonate, 4-chlorobenzenesulfonate; citrate; ethanesulfonate; fumarate;

d-gluconate; d-glucuronate; glutarate; glycolate; hippurate; hydrochloride; 2-hydroxyethanesulfonate; dl-lactate; maleate; d-malate; l-malate; malonate; d-mandelate; l-mandelate; methanesulfonate; 1,5-napthalenedisulfonate; 2-naphthalenesulfonate; phosphate; salicylate; succinate; sulfate; d-tartrate; l-tartrate; and p-toluenesulfonate.

A pharmaceutical "base addition" salt is a salt formed by reaction of the free base form of a compound of formula I with a pharmaceutical base, such as described in the Encyclopedia of Pharmaceutical Technology, editors James Swarbrick and James C. Boylan, Vol 13 (1996), "Preservation of Pharmaceutical Products to Salt Forms of Drugs and Absorption". Specific salt forms include, but are not limited to the: calcium, diethanolamine, diethylamine, ethylenediamine, lysine, magnesium, piperazine, potassium, sodium and tromethamine (Tris, Trizma) salts.

The term "agonist" includes any molecule that has affinity for the MC4 receptor, producing a measurable biological activity associated with weight loss in cells, tissues and organisms containing the MC4 receptor. In a similar manner, an "inverse agonist" includes any molecule that has affinity for the MC4 receptor, producing a decreased intrinsic activity of the cell containing the MC4 receptor and is associated with weight gain in cells, tissues, and organisms containing the MC4 receptor. The term "antagonist" includes any molecule that partially or fully blocks, inhibits, or neutralizes a biological activity of the MC4 receptor. Assays measuring such activities are well known in the art.

The term "weight loss" includes any decrease in the mass of a patient. Weight loss may include overall loss of mass by the patient or, alternatively, loss of fat mass by the patient.

The term "obesity," also called corpulence or fatness, is the excessive accumulation of body fat, usually caused by the consumption of more calories than the body uses. The excess calories are then stored as fat, or adipose tissue. Overweight, if moderate, is not necessarily obesity, particularly in muscular or large-boned individuals. In general, however, a body weight twenty percent or more over the optimum tends to be associated with obesity.

A "subject" or "patient" is a mammal, preferably a human. Nonetheless, other mammals may be subjects or patients, including companion animals such as dogs and cats, laboratory animals such as rats, mice, monkeys, and guinea pigs, and farm animals such as cows, sheep, pigs, and horses.

The term "a patient in need thereof" is a patient either suffering from the caimed pathological condition or sequela thereof or is a patient at a recognized risk thereof as determined by medical diagnosis, *i.e.*, as determined by the attending physician.

The terms "treating," "treatment," and "therapy" as used herein refer to the management and care of a patient for the purpose of combating the disease, condition, or disorder. Treating includes the administration of an MC4R agonist peptide to prevent the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder. Treating obesity therefore includes the inhibition of food intake, the inhibition of weight gain, and inducing weight loss in patients in need thereof.

Treatment may include curative therapy, prophylactic therapy, and preventive therapy. An example of "preventive therapy" is the prevention or lessened targeted pathological condition or disorder. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in whom the disorder is to be prevented.

A "therapeutically-effective amount" is the minimal amount of MC4R agonist peptide necessary to induce weight loss. An "effective amount" of the peptide administered to a subject will also depend on the type and severity of the disease or condition and on the characteristics of the subject, such as general health, age, sex, body weight and tolerance to drugs. The recipient patient's physician should determine the therapeutic dose administered in light of the relevant circumstances.

Generally, an effective minimum daily dose of an MC4R agonist peptide in the present invention will exceed about 2 μg/kg. Typically, an effective maximum daily dose will not exceed about 20 μg/kg. The exact dose may be determined, in accordance with the standard practice in the medical arts of "dose titrating" the recipient; that is, initially administering a low dose of the compound, and gradually increasing the does until the desired therapeutic effect is observed.

The peptides used in the invention may be chemically synthesized. Such methods for synthesis are well known in the art.

EXAMPLES

Example 1: Comparison of Continuous Infusion of MC4R Agonist Peptide Versus Daily Subcutaneous Injection

For this type of experiment, two solutions are prepared. First, a 5% dextrose solution is prepared by diluting 5 mL 50% dextrose solution (Biomeda) in 45 mL of sterile water for injection. This dextrose solution is subsequently referred to as "vehicle." Second, a stock solution of the MC4R agonist peptide ("P1") to be administered subcutaneously is prepared by dissolving 1.75 mg of P1 in 2 mL of the vehicle. This stock solution is then diluted to 0.088 mg/mL using that vehicle. This solution is subsequently referred to as P1sc solution. Both the P1sc solution and the vehicle are prepared fresh every three days and stored at 4°C in a sterile capped vial throughout the experiment. A separate solution of MC4R agonist peptide is prepared for continuous infusion using osmotic pumps by dissolving 11.1 mg of P1 in 3 mL of vehicle [3.7 mg/mL]. This solution is subsequently referred to as P1p solution. Ten ALZET® mini-osmotic pumps (implantable infusion pumps that continuously deliver materials to laboratory animals; Model 2002, 14-day payout at 0.5 μL/hour) are loaded using aseptic technique with either 200 μL P1p (n=5) or vehicle (n=5) solution and allowed to prime overnight in sterile 0.9% saline at 37°C in preparation for implantation into rats.

Twenty rats are selected for this experiment. Ten rats are anaesthetized briefly with isoflorane (3%, Abbott Laboratories). Each anaesthetized rat is implanted with an ALZET® pump using sterile technique. The rats are divided into four groups of five rats: two groups containing pumps and two groups with no pumps. Experimental samples are administered to the rats as follows:

Table 1. Administration scheme for an P1 study.

Group	Substance	Delivery method	Approximate daily dose (μg/kg active)
1	MC4-R peptide P1	Sustained release via pump	44
2	Vehicle	Sustained release via pump	0
3	MC4-R peptide P1	Daily subcutaneous injection	44
4	Vehicle	Daily subcutaneous injection	0

Each rat is weighed initially, and measurements of body composition are made for each animal using QNMR (quantitative nuclear magnetic resonance). Body mass is measured daily for fourteen days, and the cumulative change in body mass is calculated. Body composition is measured again at the end of the study.

Using a procedure such as that described above, results shown in Tables 2, 3, and 4, below, may be achieved.

Table 2. Change in body mass among groups.

	Mean change in body mass			
Day	Group 1	Group 2	Group 3	Group 4
1	-7.66	4.14	-0.68	2.96
2	-8.52	6.20	1.64	3.26
3	-10.12	6.70	0.46	5.68
4	-9.54	7.32	0.78	8.50
5	-12.56	8.06	1.16	10.06
6	-14.02	7.74	0.94	10.51
7	-12.86	7.88	0.80	10.90
8	-14.42	10.22	4.00	11.38
9	-14.60	9.88	2.72	14.48
10	-14.72	10.90	2.98	14.46
11	-13.04	13.86	4.28	17.24
12	-12.22	17.46	7.30	19.64
13	-9.66	18.70	9.52	20.66
14	-9.12	20.32	9.54	23.22

Table 3. Change in fat mass among groups.

	Mean fat mass			
Day	Group 1	Group 2	Group 3	Group 4
0	78.171	81.725	74.252	81.312
14	69.273	89.887	72.912	93.182
Change	-8.898	8.162	-1.340	11.870

Table 4. Change in lean mass among groups.

	Mean lean mass			
Day	Group 1	Group 2	Group 3	Group 4
0	328.609	329.489	340.206	333.134
14	330.373	344.131	353.033	344.527
Change	1.764	14.642	12.827	11.393

Additionally, the food intake of each animal (mass of food the animal eats in one day) is measured daily during the fourteen-day experiment. Results of this study are shown in Table 5, below.

Table 5. Food intake among groups (P1).

	Mean daily food intake			
Day	Group 1	Group 2	Group 3	Group 4
1	6.76	15.98	13.76	16.06
2	10.40	19.22	15.64	19.40
3	15.14	21.36	17.58	22.18
4	14.48	20.16	15.90	20.48
5	12.20	18.26	15.94	18.50
6	12.44	16.84	14.12	17.78
7	13.96	16.70	16.70	18.68
8	14.94	17.96	16.44	16.04
9	14.22	17.48	13.24	17.58
10	16.26	18.18	17.52	17.84
11	15.70	18.36	15.46	17.62
12	15.08	16.94	16.10	17.34
13	16.76	17.78	16.30	17.40
14	14.64	17.16	15.62	16.36

Continuous subcutaneous infusion of P1 in rats results in improved efficacy over single daily bolus dosing of equivalent P1 [0.044 mg/kg]. Cumulative weight loss in rats infused with P1 is significantly increased over both vehicle treated groups and rats dosed once daily. Decreased fat mass in rats continuously infused with peptide also indicates improved efficacy over daily dosing; however, the change does not reach significance between infused and daily dosed groups.

Experiments such as that described above may be performed on other MC4R agonists and for different time periods. For example, a seven-day study administering another peptide ("P2") may be performed. A stock solution of the MC4R peptide to be dose subcutaneous is prepared by dissolving 2 mg of P2 in 2 mL of the vehicle. This stock solution is then diluted 0.1 mg/mL using vehicle. This solution is subsequently referred to as P2sc solution. Both the P2sc solution and the vehicle are prepared fresh every three days and stored at 4°C in a sterile capped vial throughout the experiment. A second solution of the MC4R peptide P2 is prepared by dissolving 5 mg of P2 in 2.4 mL

of the vehicle prepared above. This solution is subsequently referred to as P2p solution. Ten ALZET® mini-osmotic pumps (Model 2001, 7-day payout at 1.0 μL/hour) are loaded using aseptic technique with either 200 μL P2 (n=4) or vehicle (n=4) solution and allowed to prime overnight in sterile 0.9% saline at 37°C in preparation for implantation into rats.

Sixteen rats are selected for this experiment. Ten rats are anaesthetized briefly with isoflorane (prepared above). Each anaesthetized rat is implanted with an ALZET® pump using sterile technique. The rats are divided into four groups of four rats: two groups containing pumps and two groups with no pumps. Experimental samples are administered to the rats as follows:

Table 6. Administration scheme for a P2 study.

Group	Substance	Delivery method	Approximate daily dose (μg/kg active)
1	MC4-R peptide P2	Sustained release via pump	50
2	Vehicle	Sustained release via pump	0
3	MC4-R peptide P2	Daily subcutaneous injection	50
4	Vehicle	Daily subcutaneous injection	0

Body mass is measured daily for seven days, and the cumulative change in body mass is calculated.

Using a procedure such as that described above, results shown in Table 7, below, may be achieved.

Table 7. Change in body mass among groups (P2).

	Mean change in body mass			
Day	Group 1	Group 2	Group 3	Group 4
1	-5.52	7.03	5.50	5.88
2	-3.95	8.20	9.63	7.92
3	-10.53	1.30	5.20	4.72
4	-8.85	3.80	5.55	6.77
5	-11.35	2.60	7.43	8.82
6	-11.75	4.90	8.65	11.05
7	-13.73	6.60	9.73	12.88

Additionally, the food intake of each animal (mass of food the animal eats in one day) is measured daily during the seven-day experiment. Results of this study are shown in Table 8, below.

Table 8. Food intake among groups (P2).

	Mean daily food intake			
Day	Group 1	Group 2	Group 3	Group 4
1	16.05	22.83	19.33	23.38
2	15.78	20.03	17.75	21.98
3	15.43	16.03	15.85	20.88
4	12.08	13.83	16.75	15.75
5	13.15	16.28	14.83	22.25
6	13.75	17.23	14.90	15.95
7	13.63	17.95	16.68	18.35

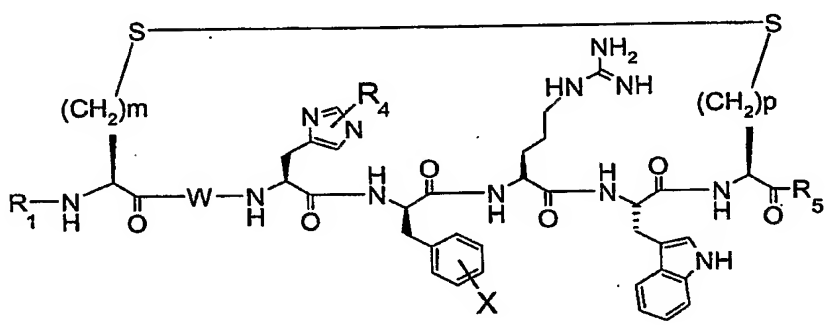
Continuous subcutaneous infusion of P2 in rats supports P1 study results. Infusion of peptide improved efficacy over single daily bolus dosing of equivalent P2 [0.05 mg/kg]. Cumulative weight loss in rats infused with P2 is significantly increased over both vehicle treated groups and rats dosed once daily.

WHAT IS CLAIMED IS:

1. A method of inducing weight loss in a patient, comprising administering by continuous infusion an effective amount of an MC4R agonist peptideto a patient in need thereof.

2. A method for treating obesity in a patient, comprising administering by continuous infusion an effective amount of an MC4R agonist peptideto a patient in need thereof.

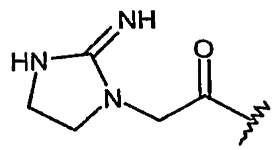
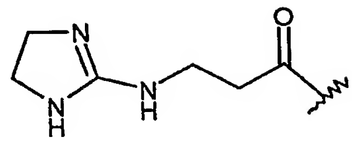
3. The method of any one of Claims 1 to 2, wherein the MC4R agonist peptide is a peptide of the formula:

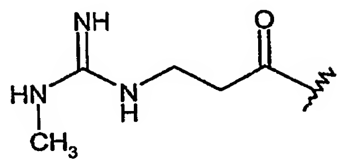
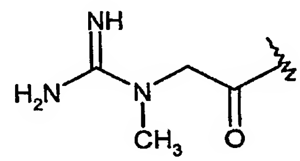


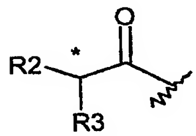
and pharmaceutically acceptable salts thereof, wherein

W is a single bond, Glu, Gln, Asp, Asn, Ala, Gly, Thr, Ser, Pro, Met, Ile, Val, Arg, His, Tyr, Trp, or Phe;

R_1 is -H, $-C(O)CH_3$, $-C(O)(CH_2)_{1-4}CH_3$, $-NH-C(NH)NH_2$, Tyr-βArg, gluconoyl-Tyr-Arg, Ac-Dab, Ac-Dap, N-succinyl-Tyr-Arg, N-propionyl, N-valeryl, N-glutaryl-Tyr-Arg, N-butyryl,

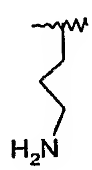
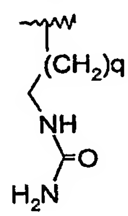
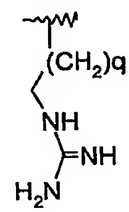
, ,

, , or

, wherein

R_2 is -H, $-NH_2$, $-NHC(O)CH_3$, $-NHC(O)(CH_2)_{1\text{-}4}CH_3$, Tyr, or -NH-Tyr-$C(O)CH_3$;

R_3 is C_1-C_4 straight or branched alkyl, Ser, Ile, Arg,

 ,  and  ;

q is 0, 1, 2, or 3;

m is 1 or 2;

p is 1 or 2;

R_4 is -H, $-CH_3$, or $-(CH_2)_{1\text{-}3}(CH_3)$;

X is -H, -Cl, -F, -Br, methyl, or methoxy; and

R_5 is $-NH_2$, -OH, glycinol, -Ser-Pro-NH_2, -Lys-Pro-NH_2, -Ser-OH, -Ser-Pro-OH, -Lys-Pro-OH, -Arg-Phe-NH_2, -GluNH_2, -NHR, or -OR, where R is $-CH_3$ or $-(CH_2)_{1\text{-}3}(CH_3)$.

4. The method of any one of Claims 1 to 2, wherein the MC4R agonist peptide is cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2, Ac-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-NH_2, Arg-cyclo[hCys-His-D-Phe-Arg-Trp-Cys]-OH,

Ac-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2, or
Ac-D-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2.

5. The method of any one of Claims 1 to 2, wherein the MC4R agonist peptide is Ac-D-Arg-cyclo[Cys-Glu-His-D-Phe-Arg-Trp-Cys]-NH_2.

Abstract of the Disclosure

The present invention provides a method of inducing weight loss in a patient, comprising continuous infusion of an effective amount of an MC4R agonist peptide into the patient. Additionally, the present invention provides a method for treating obesity in a patient, comprising continuous infusion of an effective amount of an MC4R agonist peptide into the patient.